Final Report – SERDP Project CU-1242, The Effects of Perchlorate on Developing and Adult Birds

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Contract Period: October 2001 - June 2003

"Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International."

Framework and Objectives of the Project:

The research in this proposal was designed to establish safe exposure levels for ammonium perchlorate (AP) in developing and adult birds and to develop assessment endpoints for determining the impact of perchlorate exposure in birds. The main focus of the experimental work was to evaluate AP effects using an array of endpoints that measure thyroid function (a key target of perchlorate anion), growth and development during embryonic, early posthatching and adult life. Laboratory dosing studies of two wildlife species of birds, Bobwhite quail (*Colinus virginianus*) and Mallard ducks (*Anas platyrhynchos*), were used to investigate the following objectives:

1. To establish safe exposure levels of perchlorate for embryos, chicks and adults based on the effects of AP on thyroid function, growth and development.
2. To evaluate measurements of thyroid function that may be used as assessment endpoints for determining the impact of perchlorate exposure in birds.
3. An additional objective was added following the first year In Progress review – to investigate the role of cations (sodium and ammonium) in combination with perchlorate anion.

AP has been used as an oxidizer in solid rocket fuels and is a ground and water contaminant on a number of military bases. AP is persistent, water soluble and readily ionized, thus it is highly labile in environmental waters. Perchlorate effects on thyroid function are fairly well understood in laboratory mammals and in certain human clinical contexts. Perchlorate ion is known to compete with iodide transport into the thyroid gland in vertebrates. This results in iodine deficiency and consequent reduced thyroid hormone (TH) synthesis, which depending on the level of exposure, results in organismal hypothyroidism. THs are required for normal development and growth, as well as adult function of many critical systems (e.g., central nervous system, musculoskeletal system) in all vertebrates. Thus, hypothyroidism can have severe permanent effects on developing animals and can cause functional inadequacies in adults. Because perchlorate effects on birds had not been investigated previously, our studies required fundamental investigations of both the nature and dose-response characteristics of effects resulting from perchlorate exposure in birds and of the capability and sensitivity of specific types of thyroid assessments for detecting those effects. These investigations addressed different doses and exposure times and different developmental stages (embryos, chicks, adults) in two wildlife species using multiple indicators of thyroid function, growth and development.

Bobwhite quail are a good example of an avian wildlife species that are likely to experience the full impact of the most extreme AP exposures in their native habitat. Quail are ground-dwelling birds that use local water sources and are likely to inhabit military bases. They are year-round inhabitants of a single habitat so will have sustained exposure to any

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JUN 2003		**00-00-2003 to 00-00-2003**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
The Effects of Perchlorate on Developing and Adult Birds	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Virginia Tech,Dept. of Biology,Blacksburg,VA,24061-0406**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**18**	

contaminants in their local water sources. In contrast, mallard ducks typically live near large bodies of water, are migratory and are strong fliers. Thus, these birds are less likely to encounter concentrated AP such as in small waste ponds and are less likely than quail to have sustained high AP exposure. In choosing the range of AP exposures used in our studies, we considered the following published literature. Groundwater at contaminated sites may have perchlorate ion concentrations as high as 3700 mg/L (Urbansky 1998). Recent analyses of water on a military base in Texas found concentrations of 30-31 mg/L perchlorate ion in a perchlorate holding pond and concentrations <85 μg/L at other sites. This study also found 840-7620 μg/kg perchlorate on a dry-weight basis in plants near the holding pond (Smith et al. 2001). Thus, birds may be exposed to AP in both food and water sources. Most of the attention focused on perchlorate contamination has been directed to human exposure in public water supplies (Soldin et al. 2001) from large bodies of water where perchlorate is at relatively low concentrations (5-24 μg/L in some public water supplies in California and Nevada; Urbansky 1998). In contrast, water and food utilized by ground-dwelling wildlife are from local sources so it is useful to know the nature of wildlife responses to a much wider range of exposures than may be relevant for human populations.

Approaches and Techniques in Relation to Objectives:

Objective 1: To establish safe exposure levels of perchlorate for embryos, chicks and adults. This part of the study began with range finding experiments using developing birds, which are potentially more sensitive to endocrine disruption than adults. A wide range of high AP exposures in drinking water (250-4,000 mg/L) was used in 2-week, short term experiments to determine the range of doses to be used in the more detailed, longer range studies. In the second part of the study, we extended the 2-week exposures to lower AP concentrations (below 50 μg/L) and did several series of longer range exposures. These studies were done on embryos, chicks and adults of bobwhite quail (*Colinus virginianus*) and on embryos and ducklings of Mallard ducks (*Anas platyrhynchos*), both Environmental Protection Agency approved models for studies of avian toxicology.

We evaluated organismal thyroid status (circulating concentrations of THs, thyroxine [T_4] and triiodothyronine [T_3]), activation of the HPT axis (thyroid weights; thyroid hypertrophy caused by increased pituitary thyrotropin) and thyroid hormone stores (thyroidal T_4 and T_3 content). In some studies we also measured endpoints known to be affected by TH deficiency: body growth, as indicated by body weight, and skeletal growth, as indicated by femur and tibia length. The objective of these studies was to determine safe exposure levels of perchlorate for birds, with sufficient information to establish how birds may differ from other vertebrates in their responses to this chemical.

Objective 2: To evaluate measurements of thyroid function that may be used as assessment endpoints for determining the impact of perchlorate exposure in birds.
This part of the study evaluated all results on perchlorate effects on thyroid and developmental variables, from studies in Objective 1, to determine which measurements are suitable as assessment endpoints for evaluating the effects of perchlorate exposure in birds in their natural habitat.

Objective 3: To evaluate the effects of sodium and ammonium cations in combination with the perchlorate anion. This part of the study used the same techniques as Objective 1 but quail were exposed to (1) sodium vs. ammonium perchlorate at the same perchlorate ion concentrations or (2) ammonium chloride vs. ammonium perchlorate at the same ammonium ion concentrations.

Procedures:

Bobwhite quail eggs, chicks and adults used in this study were obtained from a breeding colony in the College of Veterinary Medicine at Virginia Tech. Experimental treatment and

maintenance during the experiment were done in our animal facilities in the Dept. of Biology, Virginia Tech. Embryos were exposed by injection of AP in 25 μl of distilled water onto the air cell on day 4 of incubation, sampling was at day 21 (of the 23 day incubation period) prior to rise in thyroid hormones during the perihatch period in quail. Chicks were exposed to AP in drinking water beginning at days 3-4 posthatch. Adult studies were initiated in young adults just coming into reproductive capability. Mallard eggs and ducklings were obtained from a game bird farm in Michigan and the experimental work was done at Virginia Tech as described for quail. Mallard eggs were injected with AP onto the air cell on day 4 of incubation and sampled on day 25 of the 27-day incubation period. Mallard ducklings were exposed to AP in drinking water beginning at 3-4 days posthatch.

Animals were sacrificed, blood was collected in heparinized tubes (plasma stored at –20°C), body weight was determined to the nearest 0.01 g, and the thyroid glands were dissected out, weighed to the nearest 0.01 mg and frozen until analysis. In some experiments femur and tibia length were measured to 0.01 mm using a dial caliper.

THs were measured using a double antibody radioimmunoassay. For plasma hormone measurements, standards were prepared in hormone-stripped chicken plasma. All methods were validated according to standard endocrine procedures to insure their accurate performance on samples from each species of bird used. For measurements of the TH content of thyroid glands, the glands were digested with a bacterial Pronase to free the hormones from their storage protein (thyroglobulin), then the hormones were extracted in ethanol and the T_4 and T_3 content of the extracts was analyzed by radioimmunoassay using standards prepared in ethanol. Additional details of these methods are given in McNabb et al. [1].

Results and Interpretation:

Assessments of AP effects in birds (Objective 2)**:**

This section is discussed first because it provides useful framework for understanding the results of specific experiments.

Our studies show that the most sensitive indicator of thyroid disruption in birds is the thyroid gland T4 content (T_4 represents >95% of the thyroidal hormone stores) [4,8,9]. Thus, although birds with depleted thyroidal T_4 content aren't always overtly hypothyroid, these birds will not have normal TH reserves for responding to environmental conditions (such as cold exposure) that are associated with increases in TH demand. To our knowledge, no other laboratory has used measurements of thyroid gland TH content to assess thyroid function in the context of endocrine disruption. We developed this assay as part of our previous studies of avian thyroid development to better understand how the thyroid gland matures (see McNabb and Cheng, 1985). Previously, studies of AP effects on mammalian thyroid gland function typically have examined thyroid histology (e.g. Siglin et al. 2000; York et al. 2001a,b). However, this technique is primarily qualitative, is difficult to quantify and is very labor intensive. Thus, we chose to measure thyroidal TH content to develop a better quantitative method for assessing the state of the thyroid gland. Our work on this SERDP project indicates the measurement of thyroidal hormone content has promise as a sensitive tool for detecting thyroid disruption.

A unique feature of thyroid function, namely that the thyroid is the only endocrine gland capable of hormone storage, appears to be the key reason why thyroidal T_4 content is the most sensitive indicator and plasma TH concentrations are much less useful indicators of thyroid alterations. Thus, exposure to perchlorate or other agents that cause decreases in circulating THs, results in feedback activation of the HPT axis (increased thyrotropin release) that leads to the release of stored hormones. This release of stored hormone provides at least temporary compensation and restoration of euthyroid levels of circulating THs (Delange et al. 1996; Taurog 1996). There is evidence in the mammalian literature that sustained exposure to thyroid inhibitors can lead to a cyclic pattern of responses with temporary restoration of euthyroid levels of circulating hormone followed by the return of hypothyroid conditions (with continued exposure

to the inhibitor), renewed HPT axis activation leading to more stored hormone release, etc. (York et al. 1991a,b). The patterns of plasma THs in our study were consistent with the interpretation that such cyclic responses were occurring in our studies of AP effects in bobwhite quail. This report emphasizes T_4, the predominant thyroid hormone. Although T_3 is the more metabolically active thyroid hormone, our studies indicate that T_4 patterns give the most useful information about alterations in thyroid function in response to AP exposure.

Previous studies of thyroid disruption by perchlorate and other thyroid inhibitors (primarily on mammals) have typically measured plasma THs or assessed hypothalamic-pituitary-thyroid axis activation by measuring plasma thyrotropin concentrations or by evaluating thyroid histology. Assays for avian thyrotropin are not available and histology is very labor intensive and hard to quantify, so we used thyroid weights as our indicator of HPT axis activation (pituitary thyrotropin stimulates thyroid growth). In general, changes in thyroid weight were equivalent to measures of plasma THs in assessing thyroid disruption (both were much less sensitive indicators than thyroidal T_4 content) [1]. This result is consistent with literature which shows plasma THs, plasma thyrotropin and thyroid gland histology have similar sensitivities for detecting alterations in thyroid function in perchlorate-exposed mammals (York et al. 2001).

We also evaluated whether “downstream” target organ effects of thyroid deficiencies resulting from AP could be used to detect thyroid disruption. If such measurements could be made on intact animals these measurements would be useful for environmental monitoring. However, our studies indicate that body weight and skeletal growth variables (femur and tibia length) are very insensitive indicators of thyroid disruption. For example, in AP-exposed bobwhite chicks, body weight was unaffected and skeletal endpoints (femur and tibia growth) were altered only after relatively long (4 weeks) exposure to very high AP concentrations (e.g. 4,000 mg/L) [1]. We speculate that this is because sustained organismal hypothyroidism is necessary before end organ effects are apparent. If target organ measurements are to be used in this context, they will probably require animal sacrifice and will need to be very sensitive measures such as disturbances of molecular or biochemical events in a system such as the nervous system whose function is critical to the dynamic state of the animals.

Dose response studies to establish safe limits for AP exposure (Objective 1)**:**

Because there are no established protocols for evaluating thyroid disruption in birds, our studies addressed the effects of both AP exposure levels and exposure time. (It should be noted that protocols for evaluating AP effects on mammals also have not been established). We also needed to build a basic understanding of AP effects on thyroid function in both avian species we used to insure the appropriateness of our sampling times relative to the developmental ages studied. In addition, because of the unique storage capacity of the thyroid gland and the potential for cyclic patterns of response (see discussion in section above) there is more complexity to the timing of thyroid responses than is the case with disruption of other hormone systems.

In the summary presentation below, emphasis is placed on thyroidal T_4 content results because this measurement is the most sensitive indicator of thyroid disruption (see discussion above) but all thyroid variables measured are discussed in publications. To address a wide range of AP exposures we used a series of overlapping studies of different AP ranges. To present the wide range AP results, individual studies are combined by expressing the treatment data as % of controls within each study. Throughout our studies, dose-response data were analyzed using linear regression of either raw data or log transformed data (if the relationship was exponential). When ANOVA indicated there were differences between the thyroid responses for exposure to different AP concentrations, Fisher's Protected Least Significant Difference test was used to determine which treatments differed from control values. Probabilities of $p<0.05$ were considered indicative of statistically significant differences.

Bobwhite quail chicks:

Our most extensive studies of posthatch birds have involved 2 week AP exposures. Thyroidal T_4 content was the most sensitive measure of decreased thyroid function whereas plasma THs and thyroid gland weights were similar and much less sensitive indicators. For example, Fig. 1 indicates that decreases in thyroidal T_4 content are significantly related to AP exposures from 50μg/L to 4,000 mg/L in drinking water (Fig. 1c). However, decreases in plasma T_4 and HPT axis activation (increased thyroid weights) do not occur except at AP concentrations of =500mg/L (Fig. 1 a,b). When all 2 week studies on chicks are combined, we found a lowest observed effect level (LOEL) of 50 μg/L AP in drinking water based on thyroidal T_4 content (Fig. 2) [1,10]. (NOEL of = 25 μg/L found in two other studies). These studies suggest that birds with depleted thyroidal T_4 content will be unable to respond to environmental conditions such as cold exposure that are associated with increases in TH demand.

We also studied thyroid variables in relation to different times and AP exposures [6, 10,11]. At high AP exposures (=500 mg/L) longer exposure time (8 weeks compared to 2 weeks) leads to additional depletion of thyroidal T_4 content, decreases in plasma THs and increases in thyroid gland weights. However, at lower AP exposures (12.5 μg/L to 5 mg/L) chicks appeared to be able to partially compensate for the initial AP effects of thyroidal T_4 depletion with sustained exposure up to 8 weeks (Fig. 3). Thus, although it is often assumed that developing animals are more vulnerable to chemical exposure than adults, our results argue that at relatively low AP exposures over a limited time range (up to 8 weeks) chicks show some adaptation to sustained AP exposure. Presumably this results from increased thyroid gland size and functional capacity stimulated by pituitary thyrotropin. This should increase the iodide transport capacity of the gland in effect enhancing iodide uptake relative to the partial perchlorate inhibition at low to moderate AP exposures [10]. We speculate that in developing birds there may be greater plasticity of the thyroid gland than in adults because we saw the opposite response in our studies of long range adult quail exposures to AP (see section below).

Bobwhite quail adults:

Adult bobwhite quail tolerate higher AP exposures than do quail chicks and they are slower to develop signs of decreased thyroid function. For example, adult quail exposed to AP concentrations up to 8,000mg/L AP in drinking water for 3 weeks showed extreme variability in individual responses for all thyroid variables but did not differ significantly from controls (see Fig. 4 for thyroidal T_4 content data; no LOEL established). With continued AP exposure there were increasing thyroid effects. For example, by 5 weeks of exposure all groups exposed to =250 mg/L had significantly depleted thyroidal T_4 content and by 8 weeks all groups =50 μg/L had significantly depleted thyroidal T_4 (8 week LOEL 50 μg/L). Thus, in contrast to bobwhite chicks, which showed some compensation for the initial effects of low AP exposure when the AP exposure was sustained, bobwhite adults showed further decreases in thyroid function with increasing AP exposure time (Fig. 5) [11].

Bobwhite quail embryos:

Ammonium perchlorate is water soluble and is presumed to enter avian eggs but we know of no published data on avian egg AP content. Until experimentation with hens exposed to AP and data on AP in their eggs are available, we cannot be sure what concentrations of AP embryos may be exposed to *in ovo*. The experimental doses we put into eggs are based on tissue AP measurements from the literature and the assumption that egg AP deposition may be in proportion to the AP content of maternal tissues.

Thyroidal T_4 content of quail embryos was inversely related to AP concentration in eggs from 25 μg/kg to 150 mg/kg. Results were highly variable at the three lowest AP exposures (25, 50 and 100 μg/kg egg). At =1 mg/kg egg AP concentrations (LOEL 1 mg/kg egg AP; NOEL 0.5 mg/kg), thyroidal T_4 content was =60% that of embryos from control eggs injected with distilled water. Embryo mortality increased with AP exposure compared to controls injected with distilled water (data currently being analyzed). In a few experiments we hatched some of the AP-treated

eggs. One day old hatchlings from eggs with 50-150 mg/kg AP had thyroidal T_4 content ~50% that of hatchlings from control eggs or from uninjected eggs (Fig. 6). If we compare embryo exposure (as AP concentration in the egg) with chick exposure studies (as AP concentration in the drinking water), it appears that embryos are less sensitive to AP than chicks. However, without tissue AP data, it is not clear whether this is an appropriate comparison. Second, the increasing mortality in embryos with increasing AP exposure may be selecting out vulnerable individuals. Completion of the analysis of our embryonic data will be necessary before further interpretation of these studies.

Mallard duck development:

Before studying the effects of AP on developing mallard ducks we did a basic investigation of the pattern of thyroid development in this precocial species (Fig. 7). This work was necessary for determining when to sample in the context of development in this species which has not previously been studied with respect to thyroid function.

Mallard ducklings:

Ducklings were sampled at 1 and 2 weeks of exposure to AP in drinking water. Effects of AP on thyroid function appeared at 1 week at high AP exposures but this report will focus on the 2 week exposure data for comparison with the effects of AP on bobwhite quail. The LOEL for 2 week exposures in mallard ducklings was 5.0 mg/L for the dose range used (NOEL = 1mg/L; Fig. 8). These data suggest that thyroid function in ducklings is more resistant to AP effects than is the case for quail (note the 2 week LOEL for quail of 50 μg/L in the experiments described above). Ducks are less likely to use small local water sources with the highest AP concentrations. However, we were unable to do long range studies of AP effects on ducks within this project so it is not clear whether 2 week tests are sufficient to determine safe levels of exposure for this species [4, 5, 7].

Mallard embryos:

Decreases in thyroidal T_4 content were significantly related to *in ovo* exposure to increasing concentrations of AP (Fig. 9). Mallard embryos were more sensitive to AP exposure than were bobwhite embryos. Thyroidal T_4 content in mallard embryos was significantly reduced at 50 μg/L AP in the egg (LOEL 50 μg/L; NOEL 25 μg/L) whereas in quail embryos thyroidal T_4 content wasn't significantly decreased until 1 mg/L AP in the egg [4, 5, 7]. This difference could be related to the relative maturation patterns of the embryos in these two species. We are doing a detailed examination of the comparative data for the two species to determine if our data support this idea.

Sodium vs. Ammonium Cations with Perchlorate:

Although most perchlorate contamination originally came from the release of AP, because AP readily dissociates, it is thought that most environmental perchlorate may now exist in association with sodium (Urbansky 1998). We have done a number of studies on bobwhite chicks, one study of bobwhite embryos and one study in duck embryos to evaluate whether sodium vs. ammonium cations alter the thyroidal response to perchlorate anion. In general the results from sodium perchlorate and ammonium perchlorate, at the same perchlorate ion concentrations do not differ significantly. Surprisingly, ammonium ion, as NH_4Cl, seemed to be stimulatory to thyroid function in several experiments. We have not completed our analysis of these experiments [6].

Reversibility of Perchlorate Effects:

In human clinical medicine, where perchlorate was once used to inhibit hyperthyroidism, the effects of perchlorate have been considered to be reversible (Green 1996; Wolff 1998). In response to an Action Item question about reversibility, we did a study of reversibility of AP effects on quail chicks. Plasma T_4 concentrations were decreased after 2 weeks of exposure to 500 mg/L AP in drinking water, this effects was partially reversed by 2 weeks and fully reversed by 4 weeks on distilled water. The thyroidal T_4 content analyses from this study have not yet been done.

Perchlorate Speciation:

An Action Item requested that we address whether any effects of perchlorate are due to ClO_3 or whether all effects result from ClO_4. ClO_3 is not a competitor for the iodide symporter of the thyroid gland so although chlorate is a breakdown product present in association with AP contamination, it apparently is not playing a role in the environmental effects of perchlorate.

Conclusions and Technology Transfer Potential of These Studies:

This project addressed the data gap in understanding the effects of perchlorate exposure in birds, a key wildlife group that needs to be protected from harmful chemical exposures. This work shows that measurement of thyroidal T_4 content is the most sensitive indicator for assessing thyroid disruption and suggests that a protocol for using this measure as an assessment tool should be developed. The work establishes LOELs for AP for bobwhite quail chicks and mallard ducklings using 2 week exposures. Based on their 2 week LOEL (50 μg/L), quail living on military bases would be likely to experience thyroid disruption from drinking AP contaminated local water sources based on published data of AP measurements from a number of bases (Urbansky 1998; Smith et al. 2001). However, longer range studies suggest that 2 week exposures are not adequate for evaluating longer term effects of AP. The pattern of longer range AP effects is different in chicks and adults and further research is needed to determine the most appropriate AP exposure time for tests used in making critical decisions about safe concentrations of AP. Quail chicks show some adaptation to sustained AP exposure. In contrast, quail adults show little effect of AP short range (2 week exposure) but show increasing thyroid disruption with sustained AP exposure. Bobwhite embryos, exposed to AP *in ovo* are relatively resistant to AP-induced alterations in thyroid function so embryonic exposure may not be problematic for this species. Mallard ducklings are less sensitive to AP exposure than quail and are less likely to encounter harmful AP concentrations in the water sources they use. Mallard embryos are much more sensitive to AP than quail embryos. Tentative conclusions on our studies comparing sodium vs. ammonium cations in combination with perchlorate, suggest perchlorate effects on avian thyroid function are similar when perchlorate is combined with either of these cations.

Publications:

[1] McNabb FMA, Larsen CT and Pooler PS. 2004. Ammonium perchlorate effects on thyroid function and growth in bobwhite quail chicks. Envir Toxicol & Chem 23(4):997-1003.

Presentations:

[2] McNabb FMA, Maher RD, Jones JE, Hood SA. 2002. The effects of perchlorate on thyroid function in developing and adult bobwhite quail. Poster, Third Gordon Research Conference on Environmental Endocrine Disruptors, South Hadley, MA.

[3] McNabb FMA, Maher RD, Jones JE, Hood SA. 2002. The effects of perchlorate on thyroid function in developing and adult bobwhite quail. Presentation at Society for Environmental Toxicology & Chemistry, Baltimore, MD.

[4] McNabb FMA, Fox GA, Grasman KA. 2002. Comparison of variables for evaluating pollutant effects on thyroid function in birds. Presentation at Society for Environmental Toxicology & Chemistry, Salt Lake City, UT.

[5] McNabb, FMA. 2002. Ammonium perchlorate effects on developing and adult thyroid function in bobwhite quail and mallard ducks. Poster at SERDP Partners in Environmental Technology , Technical Symposium & Workshop, Washington, DC.

[6] McNabb FMA, Queral-Kirkpatrick LT. 2003. Perchlorate exposure level, exposure time and perchlorate-associated cation effects in birds. Presentation at Society for Environmental Toxicology & Chemistry, Austin, TX.

[7] McNabb FMA, Maher RD, Jones JE, Jang DA. 2003. Ammonium perchlorate effects on developing and adult thyroid function in bobwhite quail and mallard ducks. Presentation at Society for Integrative and Comparative Biology, Toronto, ONT.

[8] McNabb FMA. 2004. Evaluating pollutant effects on thyroid function: comparing variables across species. For presentation at Society for Integrative and Comparative Biology, New Orleans, LA.

In Press:
[9] McNabb, F. M. A. Biomarkers for the assessment of avian thyroid disruption by chemical contaminants. In Press, Invited paper for Avian Poult. Biol. Reviews.

In Manuscript:
[10] McNabb FMA, Jang D and Larsen CT. Does thyroid function in developing birds adapt to sustained ammonium perchlorate exposure? For submission to Toxicological Sciences (Endocrine Toxicology section).

[11] McNabb FMA, Jang D and Larsen CT. The effects of ammonium perchlorate exposure time on thyroid function in adult bobwhite quail.

Other manuscripts planned for the data included in this report:
Thyroidal effects of ammonium perchlorate exposure in bobwhite quail embryos. (This paper will include the basic study of thyroid development in bobwhite quail).

Thyroidal effects of ammonium perchlorate exposure in mallard embryos and ducklings. (This paper will include the basic study of thyroid development in mallard ducks).

Cation-perchlorate effects on thyroid function in developing bobwhite quail and mallard ducks.

Other Studies Cited in Text of the Report:
Delange FM, Ermans A-M. 1996. Iodine deficiency. In Braverman LE, Utiger RD, eds, *Werner and Ingbar's The Thyroid*, 7th ed., Lippincott-Raven, Philadelphia, PA, USA, pp 296-316.

Green WL. 1996. Antithyroid compounds. In Braverman LE, Utiger RD, eds, *Werner and Ingbar's The Thyroid*, 7th ed., Lippincott-Raven, Philadelphia, PA, USA, pp 266-276.

McNabb FMA, Cheng M-F. 1985. Thyroid development in Ring doves, *Streptopelia risoria. Gen Comp Endocrinol* 58:243-251.

Smith PN, Theodorakis CW, Anderson TA, Kendall RJ. 2001. Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. *Ecotoxicology* 10:305-313.

Siglin JC, Mattie DR, Dodd DE, Hildebrandt PK, Baker WH. 2000. A 90-day drinking water toxicity study in rats with the environmental contaminant ammonium perchlorate. *Toxicol Sci* 57:61-74.

Soldin OP, Braverman LE, Lamm SH. 2001. Perchlorate clinical pharmacology and human health: A review. *Ther Drug Monit* 23:316-331.

Taurog A. 1996. Hormone synthesis: Thyroid iodine metabolism. In Braverman LE, Utiger RD, eds, *Werner and Ingbar's The Thyroid*, 7th ed., Lippincott-Raven, Philadelphia, PA, USA, pp 47-81.

Urbansky ET. 1998. Perchlorate chemistry: implications for analysis and remediation. *Bioremediation Journal* 2:81-95.

Wolff J. 1998. Perchlorate and the thyroid gland. *Pharmacol Rev* 50:89-105.

York RG, Brown WR, Girard MF, Dollarhide JS. 2001a. Two-generation reproduction study of ammonium perchlorate in drinking water in rats evaluates thyroid toxicity. *Int J Toxicol* 20:183-197.

York RG, Brown WR, Girard MF, Dollarhide JS. 2001b. Oral (drinking water) developmental toxicity study of ammonium perchlorate in New Zealand white rabbits. *Int J Toxicol* 20:199-205.

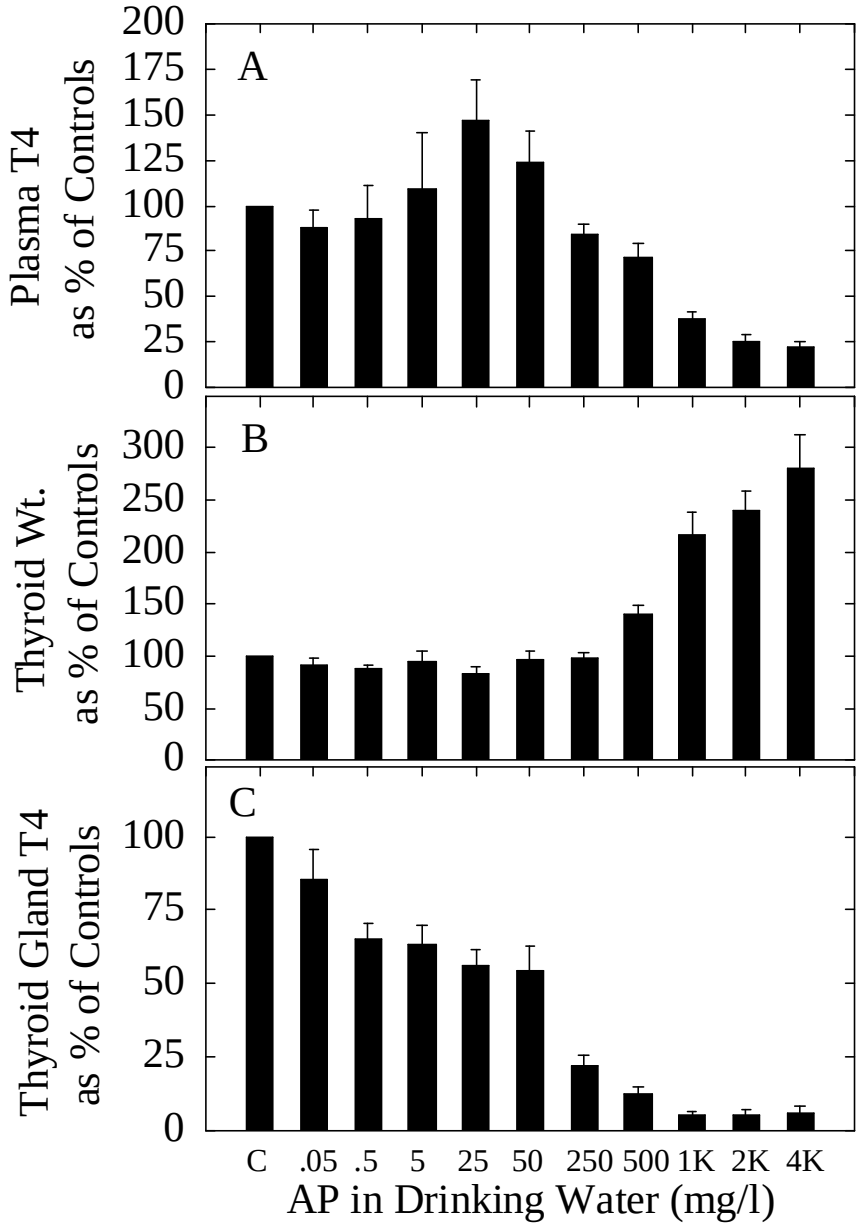

Fig. 1. Thyroid variables in bobwhite quail chicks exposed to ammonium perchlorate in drinking water for 2 weeks beginning at days 3-4 posthatch. Values are means +/- SE.

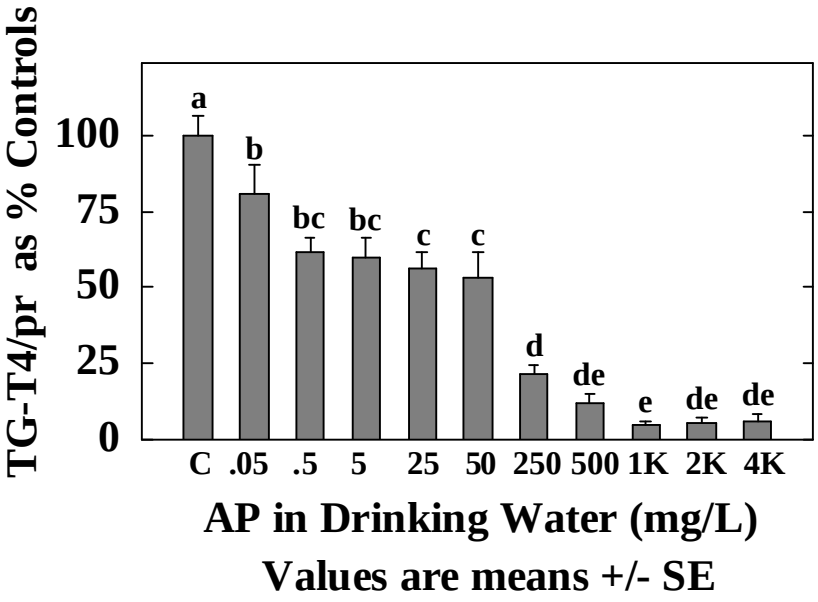

Fig. 2. Thyroidal T_4 content in bobwhite quail chicks exposed to ammonium perchlorate in drinking water for two weeks. Treatments with different letters above the bars are significantly different at $p<0.05$. Values are means +/- SE.

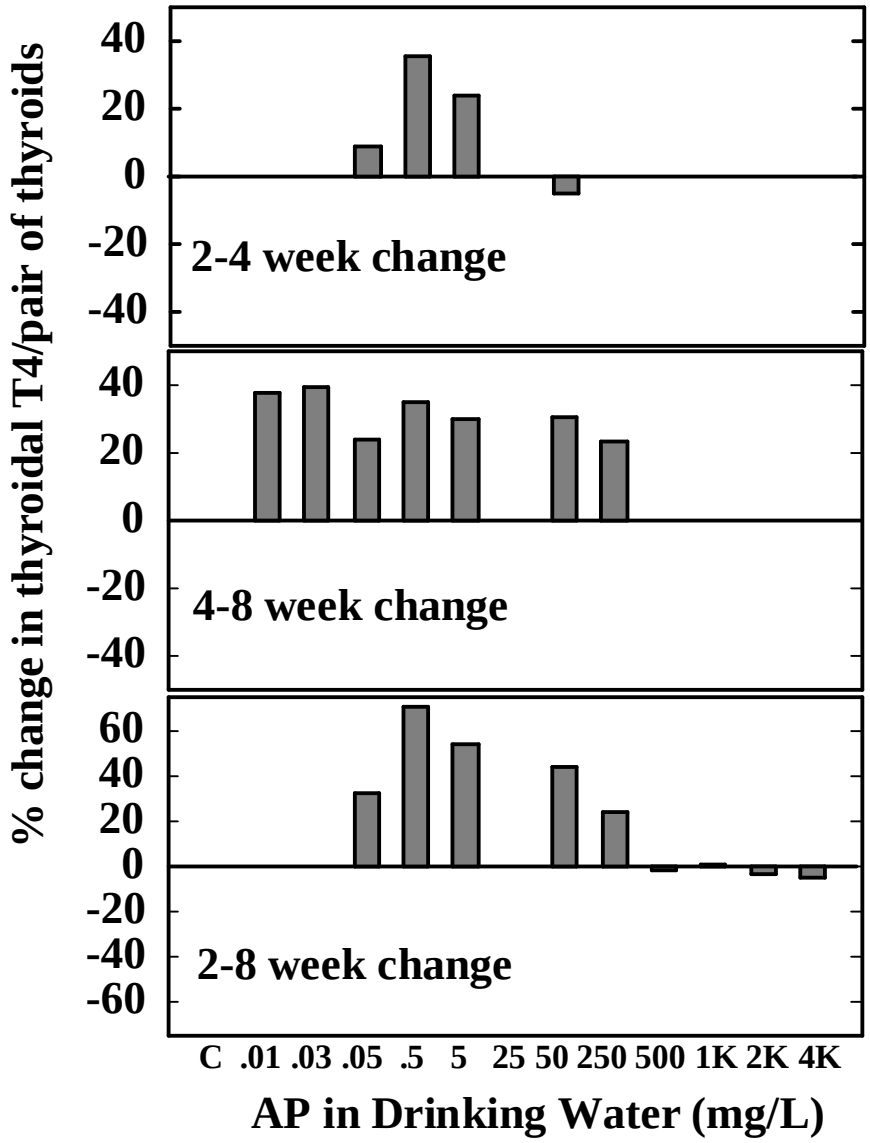

Fig. 3. Compensation in thyroid response (thyroidal T_4 content) to sustained ammonium perchlorate (AP) exposure in bobwhite quail chicks. AP decreases thyroidal T4 content during the first 2 weeks but sustained AP exposure partially reverses these effects at the lower AP concentrations used.

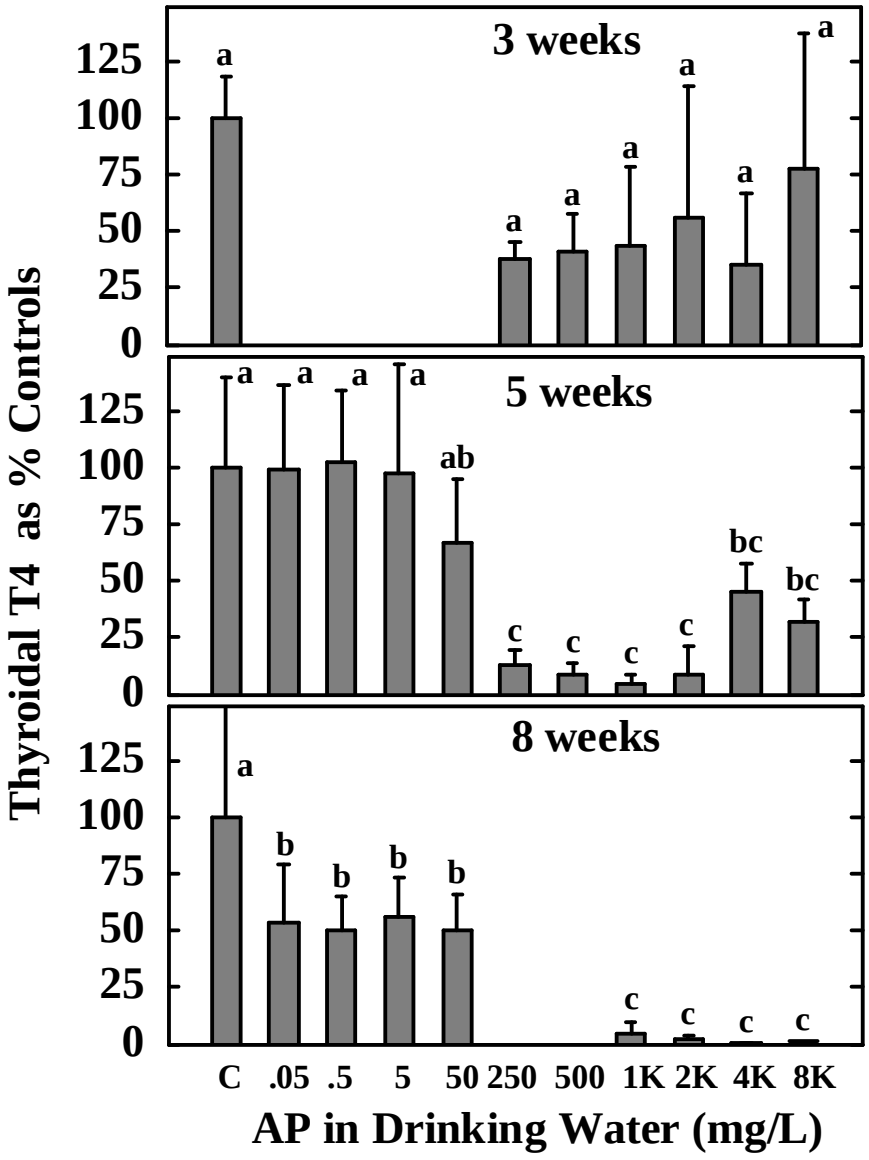

Fig. 4. Thyroidal T_4 content in adult bobwhite quail exposed to ammonium perchlorate (AP) in drinking water for 3, 5 and 8 weeks. Treatments with different letters above the bars are significantly different at $p<0.05$. Values are means +/- SE.

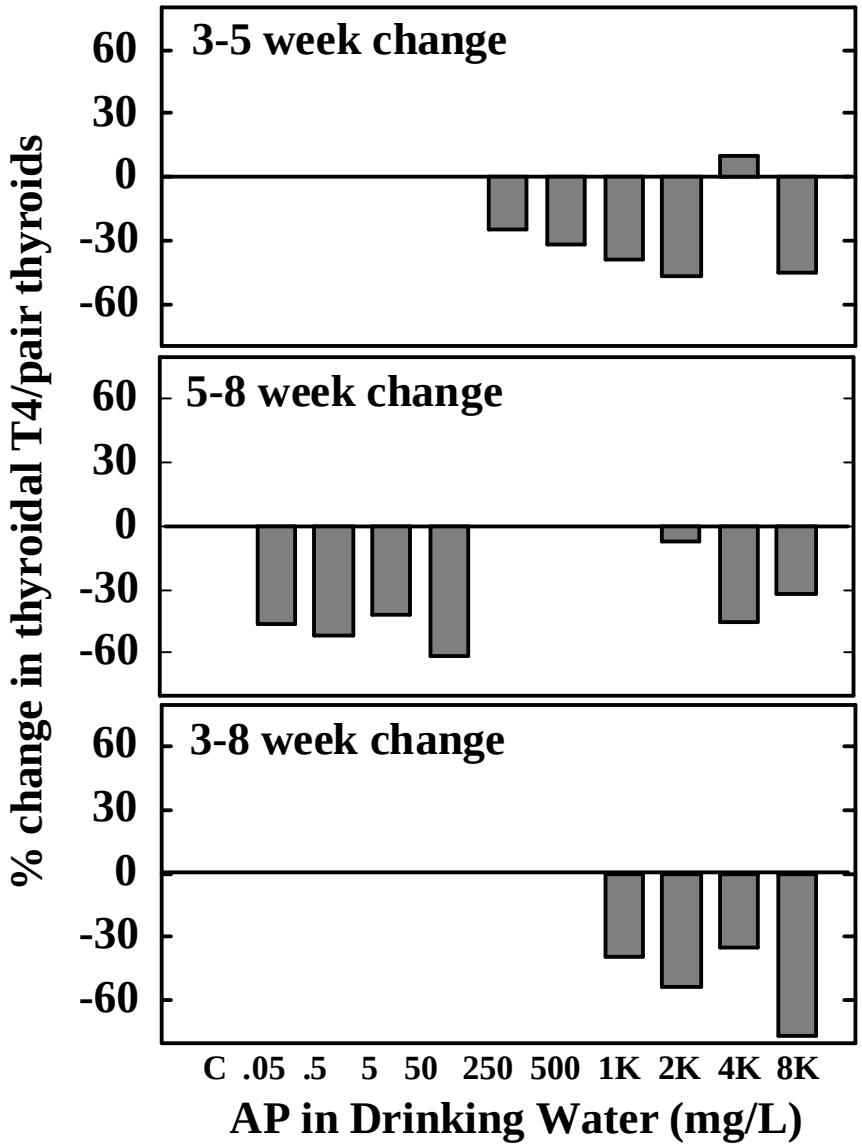

Fig. 5. Increasing thyroidal T_4 depletion with sustained AP exposure in adult bobwhite quail.

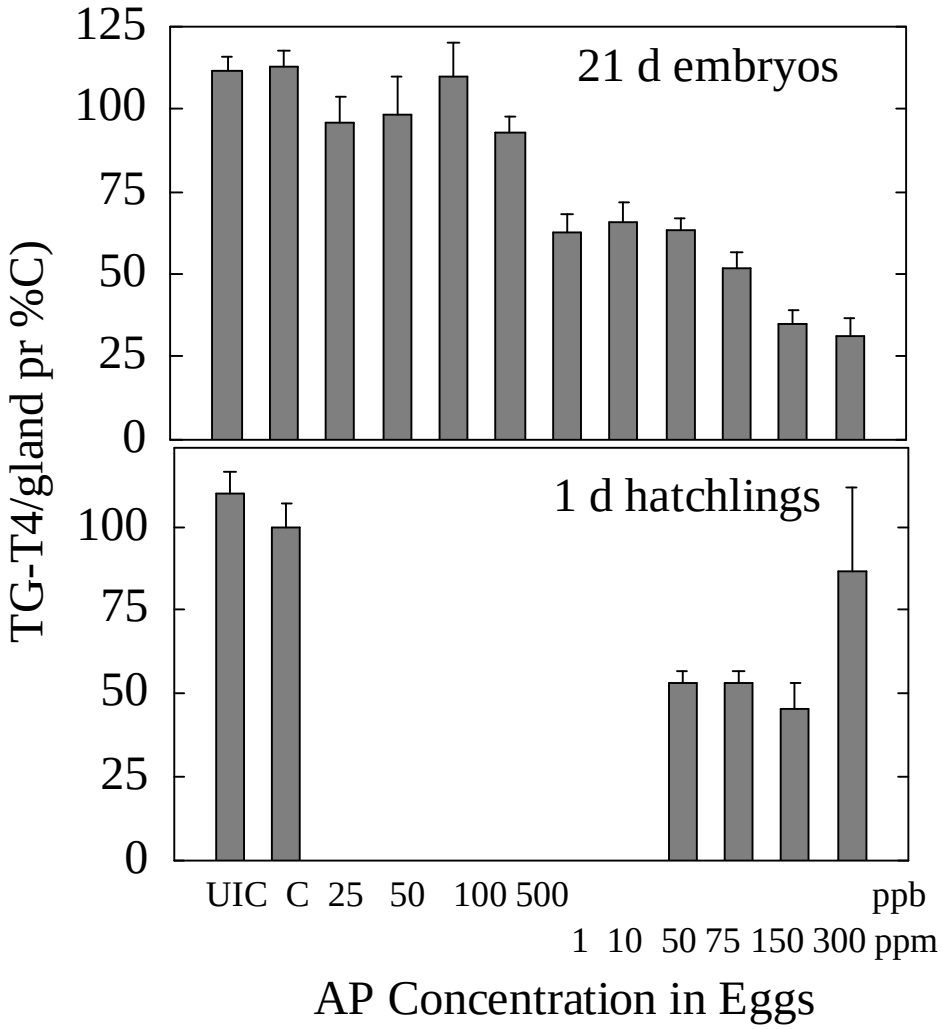

Fig. 6. Thyroidal T_4 content of bobwhite quail embryos exposed to AP *in ovo*. AP was injected onto the air cell in 25 ul of distilled water on day 4 of incubation. Embryos were sampled on day 21 of the 23-24 day incubation period. In some studies chicks were hatched and sampled after 1 day posthatch.

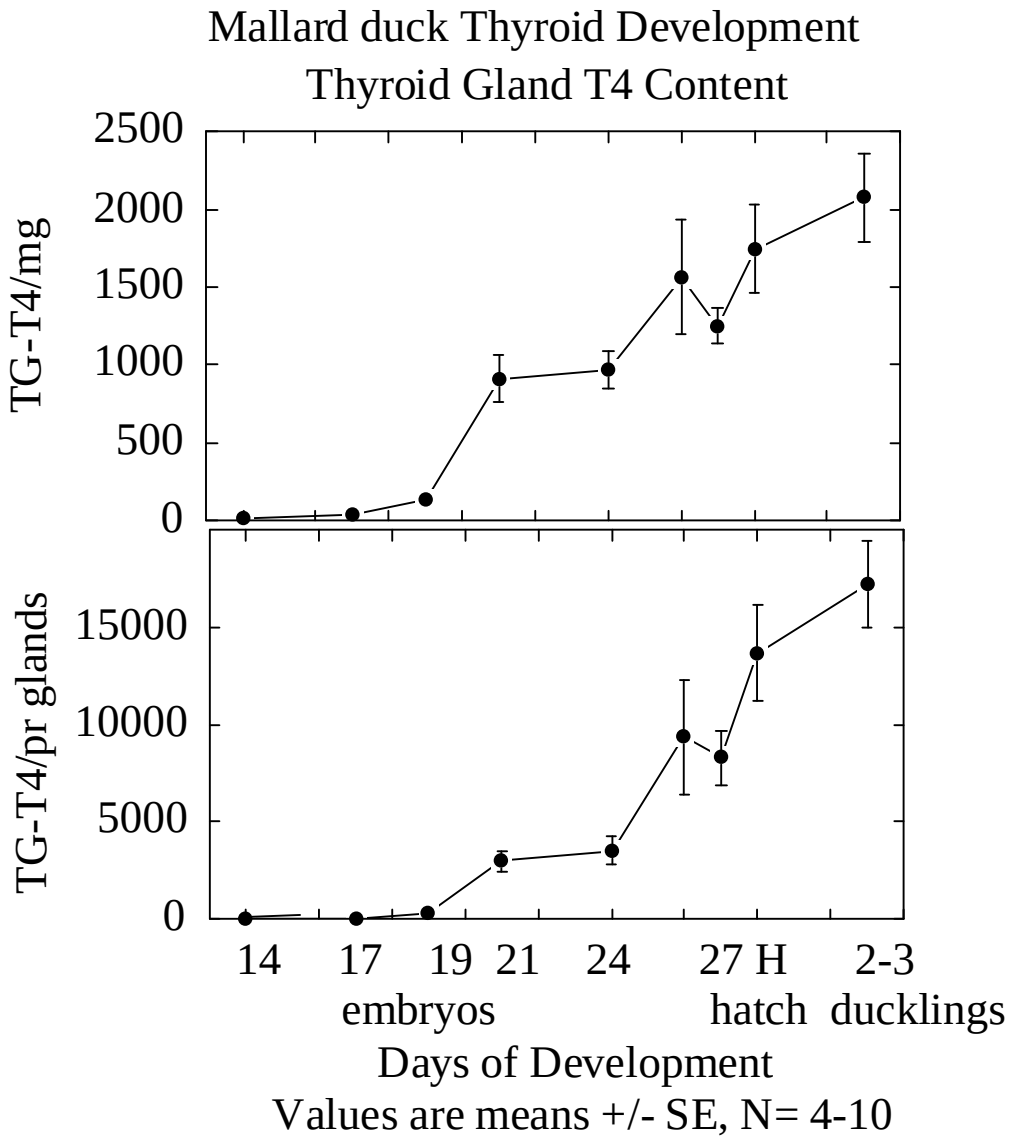

Fig. 7. Development of thyroidal T_4 stores in mallard duck embryos and early posthatch ducklings. Values are means +/- SE.

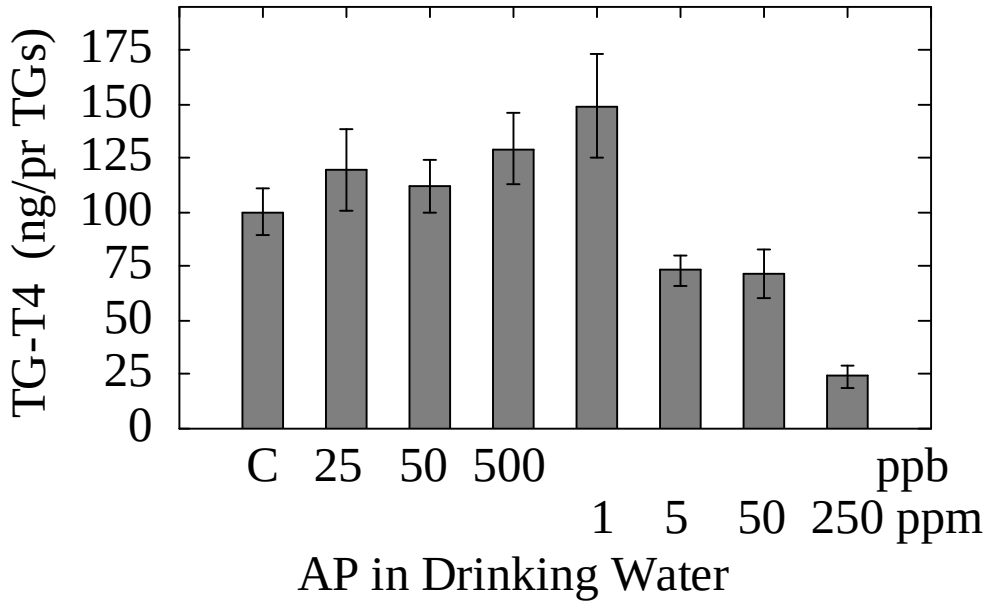

Fig. 8. Thyroidal T_4 content of mallard ducklings exposed to ammonium perchlorate in drinking water for 2 weeks. Values are means +/- SE.

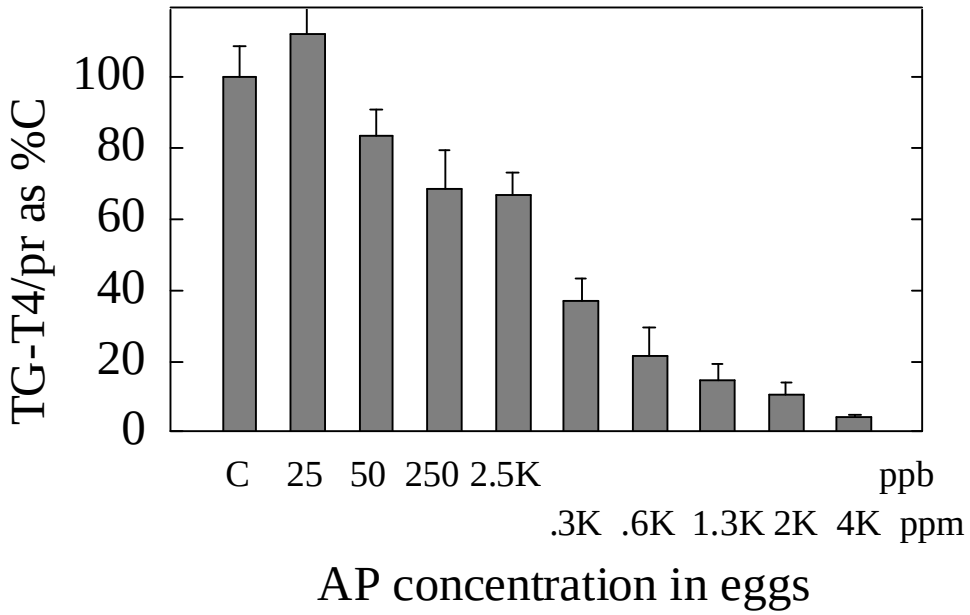

Fig. 9. Thyroidal T_4 content in mallard embryos exposed to ammonium perchlorate (AP) *in ovo*. Values are means +/- SE.